414 Rec'd PCT/PTO 28 NOV 2000

FORM PTO-1390
U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY DOCKET NUMBER
CHIR-0283

U.S. APPLICATION NO. (if known see 37 C.F.R. 1.5)
09/701453

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/11977	**28 May 1999 (28.05.99)**	**29 May 1998 (29.05.98)**

TITLE OF INVENTION
COMBINATION MEINGITIDIS B/C VACCINES

APPLICANT(S) FOR DO/EO/US
GRANOFF, Dan M.; AABERGE, Ingeborg S.; HANEBERG, Bjorn; HOLST, Johann and RUFF, Howard

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1: XX This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. _ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. _ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. XX A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. XX A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. _ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. XX has been transmitted by the International Bureau.
   c. _ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. XX A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. _ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. _ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. _ have been transmitted by the International Bureau.
   c. _ have not been made; however, the time limit for making such amendments has NOT expired.
   d. _ have not been made and will not be made.

8. _ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. _ An oath or declaration of the inventor(s) 35 U.S.C. 371(c)(4).

10. _ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. _ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. _ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. XX A FIRST preliminary amendment.
    _ A SECOND or SUBSEQUENT preliminary amendment.

14. _ A substitute specification.

15. _ A change of power of attorney and/or address letter.

16. XX Other items or information: **Return Receipt Postcard**

EXPRESS MAIL Mailing Label No. **EL531174075US**
Date of Deposit: **November 29, 2000**
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231
MAILER **Robert Galonsky**
SIGNATURE Robert Galonsky

U.S. APPLICATION NO. (if known 37 C.F.R. 1.5) 09/701453	INTERNATIONAL APPLICATION NO. PCT/US99/11977	ATTORNEY DOCKET NUMBER CHIR-0283

17. _ The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1) - (5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... **$1,000.00**

International preliminary examination fee (37 CFR 1.482 not paid to USPTO but International Search Report has been prepared by the EPO or JPO..... **$860.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... **$710.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... **$690.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... **$100.00**

	CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of $130.00 for furnishing the oath or declaration later that _ 20 _ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total claims	16 - 20 = 0	0	X $18.00	$ 0	
Independent Claims	1 - 3 = 0	0	x $80.00	$ 0	
Multiple dependent claims(s) (if applicable)			+ $270.00	$	
			TOTAL OF ABOVE CALCULATIONS =	$	
___ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.				$	
			SUBTOTAL =	$ 860.00	
Processing fee of **$130.00** for furnishing the English translation later the _ 20 _30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$	
			TOTAL NATIONAL FEE =	$ 860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property			+		
			TOTAL FEES ENCLOSED =	$ 860.00	
				Amount to be: refunded	$
				charged	$

a. X A check in the amount of $ 860.00 to cover the above fee is enclosed.

b. _ Please charge my Deposit Account No. 23-3050 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. X The Commissioner if hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-3050. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Paul K. Legaard
NAME

38,534
REGISTRATION NUMBER

DOCKET NO.: CHIR-0283 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Granoff, Aaberge, Haneberg, Holst, and Raff

Serial No.: **Not Yet Assigned** Group Art Unit: **Not Yet Assigned**

Filed: **Herewith** Examiner: **Not Yet Assigned**

For: **COMBINATION MENINGITIDIS B/C VACCINES**

"Express Mail" Label No. **EL531174075US**
Date of Deposit **November 29, 2000**

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Robert Galonsky
Typed Name: **Robert Galonsky**

Assistant Commissioner for
Patents
Washington, D.C. 20231

Dear Sir:

PRELIMINARY AMENDMENT

This Preliminary Amendment is filed along with the U.S. National Phase filing of PCT/US99/11977, filed May 28, 1999. Applicants request that the following amendments to the claims be entered prior to examination on the merits.

In the claims:

Please amend claims 15 and 16 as indicated below.

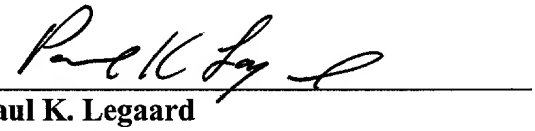
15. (Amended) A vaccine comprising an immunogenic composition of [any one of claims 1-7] claim 1.

16. (Amended) A method of vaccinating an individual comprising administering to said individual an immunogenic composition of [any one of claims 1-7] claim 1.

REMARKS

International application PCT/US99/11977 was filed on May 28, 1999. Applicants file herewith items concerning a filing under 35 U.S.C. §371. Claims 15 and 16 have been amended. Applicants reserve the right to amend claims 15 and 16 to be dependent on any of claims 1-7. No new matter has been added.

Respectfully submitted,



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Date: **November 29, 2000**

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COMBINATION MENINGITIDIS B/C VACCINES

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to U.S. provisional application Serial Numbers 60/087,351 filed May 29, 1998 and 60/106,446 filed October 30, 1998, each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to combination immunogenic compositions and vaccines for *Neisseria meningitidis* B and C and to methods of inducing an immune response by administering the same.

BACKGROUND OF THE INVENTION

Serogroup B and C strains of *Neisseria meningitidis* (Nm) together account for the majority of invasive diseases in Europe and the United States. Vaccines against individual Nm serogroups are presently available. The NIPH (National Institute of Public Health of Norway) NmB vaccine is safe, elicits strain-specific immunity in children and adults, and is efficacious in preventing NmB disease in adolescents. This vaccine has been typically combined with meningococcal C polysaccharide vaccine and given with alum. The plain polysaccharide vaccine component, however, is not effective in infants and young children. The Chiron NmC conjugate (conj.) vaccine is also safe, elicits high titers of serum bactericidal antibody in infants vaccinated as young as two and three months of age, and

induces immunologic B cell memory to the unconjugated NmC polysaccharide. Since both serogroups cause disease, a combination vaccine which induces an immune response to both serogroups would be highly advantageous.

SUMMARY OF THE INVENTION

In one aspect, the present invention relates to an immunogenic composition or vaccine comprising NmC oligosaccharides conjugated to a carrier protein, NmB outer membrane proteins, and a carrier. In a preferred embodiment, the carrier protein is CRM_{197}, a non-toxic diphtheria toxin, the NmB outer membrane proteins are presented as proteoliposomic vesicles, and the carrier is aluminum hydroxide or MF59.

In another aspect, the present invention relates to a method of inducing an immune response to NmB and NmC, or vaccinating, comprising the administration of an immunologically effective amount of an immunogenic composition comprising NmC oligosaccharides conjugated to a carrier protein, NmB outer membrane proteins, and a carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B summarize NmB IgG and NmC IgG antibody titers, respectively, as determined by ELISA.

Figures 2A and 2B summarize of titers of serum bactericidal antibody to NmB and NmC, respectively.

Figure 3 summarizes the comparison of antibody ratios to NmB and NmC induced by the combination vaccine in MF59 adjuvant vs. Alum.

Figure 4 summarizes the comparison of antibody ratios to NmB and NmC induced by the combination vaccine vs. the respective monovalent vaccine.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A combination vaccine for NmB and NmC which induces an immune response to both serogroups that is not significantly different from the immune response induced by each serogroup alone is described. The immunogenicity of the NIPH NmB vaccine (referred to herein as "NmB" or "MenB" vaccines) and the Chiron NmC conjugate vaccine (referred to herein as "NmC conj." or "MenC conj.), alone, in combination, and in combination with

the adjuvant MF59 is described herein.

The practice of the present invention will employ, unless otherwise indicated, conventional methods of immunology and microbiology. Such techniques are explained fully in the literature. See, e.g., *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.) and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., Blackwell Scientific Publications).

As used herein, the term "immunogenic" refers to material which induces the production of antibody upon administration to a vertebrate, including humans.

As used herein, the term "carrier" refers to a pharmaceutically acceptable component other than the NmB or NmC immunogenic component. The carrier can be organic, inorganic, or both. Suitable carriers well known to those of skill in the art and include, without limitation, large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes) and inactive virus particles. The carrier can also function as an immunostimulatory agent, e.g., adjuvant. Suitable adjuvants are well known to those of skill in the art.

As used herein, the term "immunologically effective amount," means the administration of that amount, either in a single dose or as part of a series, that is effective for inducing the production of antibody for either the treatment or prevention of disease. This amount will vary depending upon a variety of factors, including the physical condition of the subject, and can be readily determined by someone of skill in the art.

As used herein, the term "vaccine" means an immunogenic composition which is able to induce a microbicidal immune response. Preferably, the vaccines of the present invention elicit a bactericidal antibody response.

The present invention is directed, in part, to immunogenic compositions which induce an immune response to both Meningitidis B and C. In preferred embodiments of the invention, the immunogenic composition comprises NmB outer membrane protein, and NmC oligosaccharide conjugated to a first carrier.

The NmB protein preferably comprises partially purified outer membrane proteins from strain 44/76 (B15:P1.7, 16:L3,7,9). The partially purified outer membrane proteins are preferably present as proteoliposomic vesicles as a result of the extraction process

using deoxycholate. The dosage of NmB is expressed in μg of protein. Preferably, the NmB immune composition/vaccine components can be obtained from the National Institute of Public Health of Norway (NIPH). The NmB/alum vaccine comprises 0.05 mg/ml NmB protein, 3.33 mg/ml Al $(OH)_3$ (alum), and 0.10 mg/ml thiomersalsodium.

The Chiron oligosaccharide represents NmC polysaccharide fragments of from preferably about 12 to about 22 repeating units. Preferably, the NmC oligosaccharide is conjugated to a first carrier. The dosage of NmC conjugate or polysaccharide is expressed in μg of sialic acid. An NmC vaccine containing unconjugated polysaccharide (referred to herein as "NmC polysaccharide" or "MenC Ps") can also be used. MenC Ps is a crude isolate comprising polysaccharides preferably from about 60 to about 80 repeating units.

In preferred embodiments of the invention, the first carrier is a protein, polysaccharide, polylactic acid, polyglycolic acid, polymeric amino acids, amino acid co-polymer, lipid aggregate, or inactive virus particle. More preferably, the first carrier is a protein. Most preferably, the first carrier is CRM_{197}. Ten μg of oligosaccharide to 12.5-33 μg CRM_{197} (i.e., to maintain a oligo/protein ratio of from about 0.3 to about 0.8) is preferably used per dose. More preferably, about 20 μg of CRM_{197} can be used.

In preferred embodiments of the invention, the immunogenic composition comprises a second carrier, preferably, aluminum hydroxide (alum) or MF59. Alum can be obtained from Superfos, Bedbaek, Denmark, and is a 3% solution. When present, about 1 mg to about 1.67 mg of alum is used per dose. MF59 is a micro-fluidized emulsion of squalene in water that has been shown to be safe and to augment serum antibody responses to a variety of investigational vaccines. MF59 comprises about 5% squalene, 0.5% Tween 80 and about 0.5% Span 85. The adjuvant MF59 is described in PCT publication No. WO 90/14837, incorporated herein by reference in its entirety. MF59 can be made according to the procedures described in, for example, Ott *et al.*, *Vaccine Design: The Subunit And Adjuvant Approach*, **1995**, M.F. Powell and M.J. Newman, Eds., Plenum Press, New York, p. 277-296; Singh *et al.*, *Vaccine*, **1998**, *16*, 1822-1827; Ott *et al.*, *Vaccine*, **1995**, *13*, 1557-1562; and Valensi *et al.*, *J. Immunol.*, **1994**, *153*, 4029-39, the disclosures of which are incorporated herein by reference in their entirety.

The immunogenic composition of the invention will employ an immunologically effective amount of the antigens. That is, there will be included an amount

of antigen which, in combination with the adjuvant, will cause the subject to produce a specific and sufficient immunological response, preferably a T or B lymphocyte response, so as to impart protection to the subject from the subsequent exposure to *Neisseria*.

No single dose designation can be assigned which will provide specific guidance for each and every antigen which can be employed in this invention. The effective amount of antigen will be a function of its inherent activity and purity and is empirically determined by those of ordinary skill in the art via routine experimentation.

The immunogenic compositions according to the present invention comprise an immunostimulatory amount of *Neisseria* antigen. An immunostimulatory amount is that amount which is sufficient to induce a measurable humoral or cellular immune response. For example, the immunogenic compositions of the present invention comprise about 1 nanogram to about 1000 micrograms of antigen or about 10 nanograms to about 800 micrograms of antigen. In some preferred embodiments, the immunogenic compositions contain about 0.1 to about 500 micrograms of antigen. In some preferred embodiments, the immunogenic compositions contain about 1 to about 350 micrograms of antigen. In some preferred embodiments, the immunogenic compositions contain about 25 to about 250 micrograms of antigen. In some preferred embodiments, the immunogenic compositions contain about 100 micrograms of antigen. One skilled in the art can readily formulate an immunogenic composition comprising any desired amount of antigen, which can be empirically determined by those of ordinary skill in the art via routine experimentation. The immunogenic compositions can be conveniently administered in unit dosage form and can be prepared by any of the methods well known in the pharmaceutical art, for example, as described in Remington's Pharmaceutical Sciences (Mack Pub. Co., Easton, PA, 1980), the disclosure of which is incorporated herein by reference in its entirety.

The present invention is also directed to vaccines comprising any of the immunogenic compositions described above.

The present invention is also directed to methods of inducing an immunologic response to NmB and NmC comprising administering an immunologically effective amount of an immunogenic composition described above to a human. Administration can be by any mode known to those skilled in the art including by oral, parenteral, pulmonary, transdermal, rectal, intraperitoneal, intramuscular, or subcutaneous routes.

The invention is further illustrated by way of the following examples which are intended to elucidate the invention. The foregoing examples are meant to illustrate the invention and are not to be construed to limit the invention in any way. Those skilled in the art will recognize modifications that are within the spirit and scope of the invention. All references cited herein are hereby incorporated by reference in their entirety.

EXAMPLES

Example 1: ELISA results

Groups of guinea pigs (n=15 animals) were assigned to receive one of the following vaccines set forth in Table 1:

Table 1

Group	Components	Amount per dose
Group 1	NmC conj./alum	10 μg /1 mg
Group 2	NmB/alum	25 μg/1 mg
Group 3	NmC polysaccharide/NmB/alum	10 μg /25 μg /1 mg
Group 4	NmC conj./NmB/alum	10 μg/25 μg/1 mg
Group 5	NmC conj./NmB/MF59	10 μg /25 μg /0.5 ml.

Group 6 (n=5) comprised control animals that received alum alone.

Eighty guinea pigs were randomized into the groups set forth above and received one of six vaccine combinations. For the data presented in Table 2, each animal received two injections, IM, separated by 28 days. Serum samples were obtained prior to each injection, and 18 days after the second injection. For the data presented in Figures 1A and 1B, each animal received two immunizations separated by six weeks. Each dose consisted of two 0.25 ml IM injections. Serum samples were obtained immediately prior to each injection, and 14 or 18 days after the second injection.

Serum samples were assayed for IgG anticapsular antibody concentrations to NmC (Table 2 and Figure 1A) and for IgG anti-outer membrane vesicle antibody concentrations to NmB by ELISA (Figure 1B). The ELISA data were generated in a representative assay of individual animal sera (Table 2) and also expressed as averages from a plurality of assays (Figures 1A and 1B). The summary ELISA data set forth in Table 2 are, therefore, expressed as geometric means.

For the ELISA, MCPS-ADH (NmC polysaccharide-adipic acid dihydrazide) conjugate or outer membrane vesicle (OMV) components was coated onto polystyrene microtiter plates overnight at 4°C, 1 μg/ml, 100 μl/well. On each coated plate, 100 μl/well of each of a reference standard (i.e., pooled guinea pig serum), a positive control, a negative control, and the serum samples were two-fold serially diluted in a buffer containing 75 μM ammonium thiocyanate, and incubated for two hours at room temperature. Rabbit anti-guinea pig IgG antibody conjugated to peroxidase was added to the wells (100 μl/well). After 2 hours, the colorimetric substrate 3,3',5,5', Tetramethylbenzidine (TMB) (100 μl/well) was added, and the color was developed for 15 minutes. The levels of antibodies to MCPS ant to OMV present in the controls and samples were obtained from a standard curve using the reference standard which has an assigned value of 100 ELISA units/ml. The results are shown in Table 2 and Figures 1A and 1B.

The results summarized in Table 2 and Figures 1A and 1B reveal that the combination vaccine was immunogenic, as measured by NmB and NmC IgG antibody titers, respectively. Figure 1A shows that a specific anti-meningococcal B antibody response was induced by the vaccine combinations comprising NmB. Figure 1B shows that a specific anti-meningococcal C antibody response was induced by the vaccine combinations comprising NmC. In particular, the antibody response induced by the combination of the NmC conjugate and NmB in the presence of MF59 adjuvant (Group 5) was significantly greater than the antibody response induced by either the NmC conjugate alone (Group 1) or the combination of the NmC conjugate and NmB in the presence of alum (Group 4). When the adjuvant MF59 was present, the antibody titer for the combination vaccine increased approximately six-fold.

Table 2: IgG MenC Antibody Responses (GMT)

		SCN Assay	
Vaccine	Adjuvant	Post-1	Post-2
MenC Conj.	Alum	20.3	155
MenB	Alum	<1	<1
MenC Ps + MenB	Alum	<1	1.5

MenC Conj. + MenB	Alum	9.5	71
MenC Conj. + MenB	MF59	15.2	426
none	Alum	<1	<1

Example 2: Bactericidal Titers

Serum samples were tested for complement-mediated bactericidal titers to MenC strain 60E and MenB strain 44/76. Bactericidal titers were assayed on pooled sera from each group. Bactericidal data were generated using human complement.

Components of the assay (i.e., buffer, antibody, complement, and bacteria) were added to sterile, 96-well tissue culture plates with lids (Nunc # 167008). The plates were maintained at room temperature during the assay. To each well, 50 µl Gey's buffer (Gibco) containing 1% RIA Grade BSA (Sigma), 25 µl of the diluted test antibody, 25 µl of bacteria diluted 1:8000 in Gey's buffer/1% BSA, were sequentially added. Control wells include 1) Gey's buffer/1% BSA and bacteria alone (to determine if the organisms are viable in the diluent alone); 2) a time 0 control containing 75 µl buffer, 25 µl heat-inactivated (56°C, 30 min.) human complement, and 25 µl bacteria; and 3) a toxicity control testing the complement at 20% and 40% with buffer and bacteria to verify that the complement source is non-toxic to the test strain. All antibody samples (at the highest concentration assayed) were also tested with heat-inactivated complement to show that a decrease in colony forming units (cfu) in the presence of antibody is complement dependent. After all reagents were added, 22 µl was taken from each control well and plated onto Mueller-Hinton agar plates by allowing the sample to run from the top to the bottom of the plate, to determine the cfu in the well at 0 min. The microtiter plates were then covered and sealed with parafilm, and rotated gently for 1 hour at 37°C in a 4% CO_2 incubator. The plates were then removed, and a 22 µl sample from each well plated on Mueller-Hinton agar. The culture plates were incubated for about 18 hours at 37°C, with 4% CO_2. The colonies were counted, and % survival determined for each test well: % survival = ([cfu of sample well at 60 min]/[cfu in the heat inactivated complement control well at time 0 min.]) x 100. Bactericidal titers reported are those which resulted in 50% survival. Results from a single experiment are presented in Table 3. Results are also

presented in Figures 2A and 2B, with Figure 2B representing average titers from a plurality of experiments.

As the results summarized in Table 3 reveal, the combination vaccine elicited high titers of serum bactericidal antibody for both NmB and NmC. Bactericidal NmC antibody titer was slightly higher for the combination vaccine using MF59 as the carrier, but there was essentially no effect on bactericidal NmB titer using MF59. Interestingly, two- to five-fold higher NmB bactericidal titers were obtained with the combination vaccine than with the NmB vaccine alone. Figure 2A demonstrates that the antibodies directed to meningococcal B induced by the vaccine combinations comprising NmB were bactericidal. Figure 2B demonstrates that the antibodies directed to meningococcal C induced by the vaccine combinations comprising NmC conjugate were also bactericidal.

Table 3

	NmC (1/titer)			NmB (1/titer)		
Group Vaccine	Pre	Post-1	Post-2	Pre	Post-1	Post-2
NmC conj. + Alum	<5	80	>3375	<5	<5	<5
NmB + Alum	<5	<5	15	<5	15	800
NmC Ps + NmB + Alum	<5	<5	30	<5	25	1500
NmC Conj. + NmB + Alum	<5	25	2000	<5	25	5000
NmC Conj. + NmB + MF59	<5	50	>3375	<5	25	4000
Alum	<5	<5	<5	<5	<5	<5

Example 3: Comparison of Alum and MF59 Adjuvants

Serum from the animals described above in Figures 1A and 1B were compared and MenC and MenB antibody responses generated by NmB/NmC conj. in either alum or MF59 adjuvant were detected as described above in Examples 1 and 2. The results, shown in Figure 3, demonstrate that the antibody response to meningococcal C was approximately

6-fold greater in vaccines comprising MF59 adjuvant.

Example 4: Comparison of Antibody Responses Generated by Combination Vaccine to Monovalent Vaccines

Serum from the animals described above in Figures 1A and 1B were compared and MenC and MenB antibody responses generated by NmB/NmC conj. were compared with the antibody responses generated by either the NmB vaccine alone or the NmC conj. alone in alum as described above in Examples 1 and 2. The results, shown in Figure 4, demonstrate that there is no significant difference in the antibody responses to the components of the NmB/NmC conj. vaccine compared to the responses induced by the respective monovalent vaccines (either NmB or NmC conj.).

WHAT IS CLAIMED IS:

1. An immunogenic composition comprising NmC oligosaccharide conjugated to a first carrier and NmB outer membrane protein.

2. The immunogenic composition of claim 1 wherein said first carrier is selected from the group consisting of protein, polysaccharide, polylactic acid, polyglycolic acid, polymeric amino acids, amino acid co-polymer, lipid aggregate, and inactive virus particle.

3. The immunogenic composition of claim 2 wherein said first carrier is a protein.

4. The immunogenic composition of claim 3 wherein said first carrier is CRM_{197}.

5. The immunogenic composition of claim 1 the NmB outer membrane protein is presented as proteoliposomic vesicles.

6. The immunogenic composition of claim 1 wherein said composition comprises a second carrier.

7. The immunogenic composition of claim 6 wherein said second carrier is aluminum hydroxide or MF59.

8. A method of inducing an immunologic response to NmB and NmC comprising administering an immunologically effective amount of an immunogenic composition of claim 1.

9. The method of claim 8 wherein said first carrier is selected from the group consisting of protein, polysaccharide, polylactic acid, polyglycolic acid, polymeric amino acids, amino acid co-polymer, lipid aggregate, and inactive virus particle.

10. The method of claim 9 wherein said first carrier is a protein.

11. The method of claim 10 wherein said first carrier is CRM_{197}.

12. The method of claim 8 the NmB outer membrane protein is presented as proteoliposomic vesicles.

13. The method of claim 8 wherein said composition comprises a second carrier.

14. The method of claim 13 wherein said second carrier is aluminum hydroxide or MF59.

15. A vaccine comprising an immunogenic composition of any one of claims 1-7.

16. A method of vaccinating an individual comprising administering to said individual an immunogenic composition of any one of claims 1–7.

Figure 1A

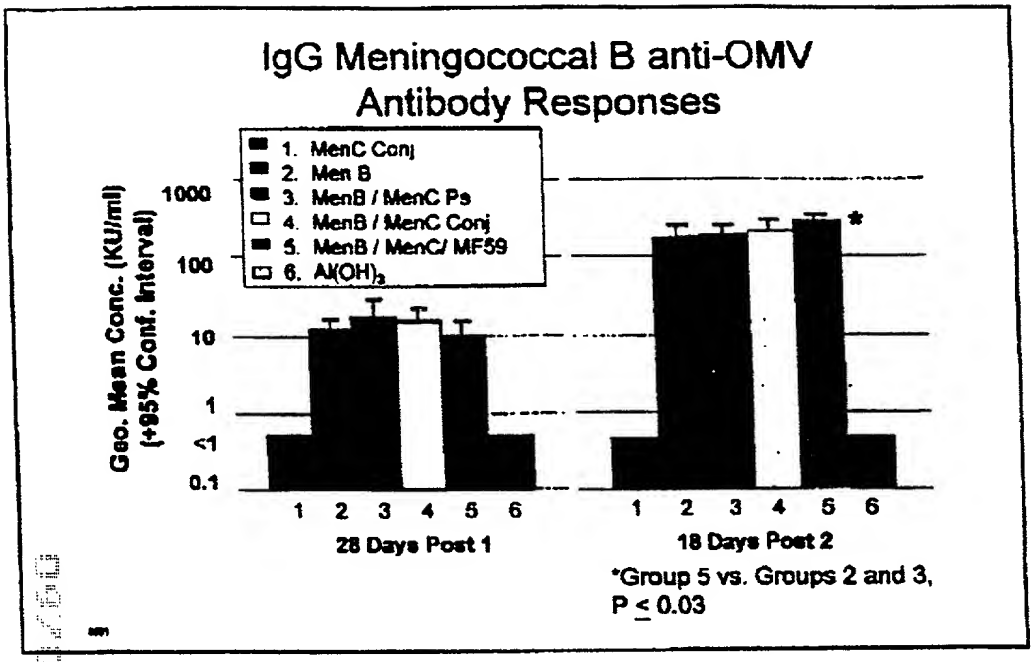

Figure 1B

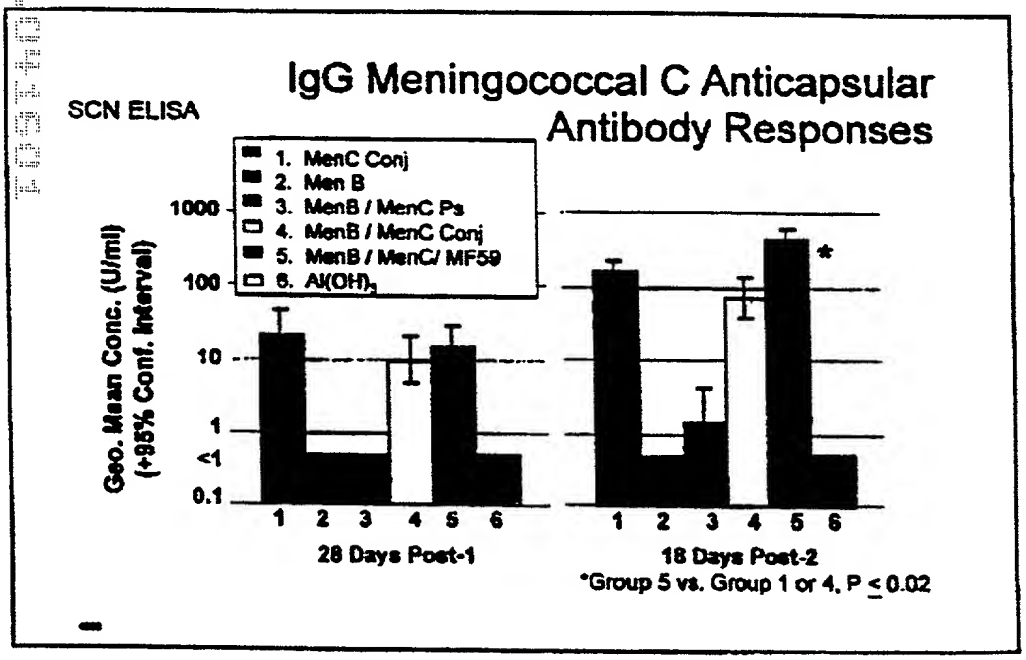

Figure 2A

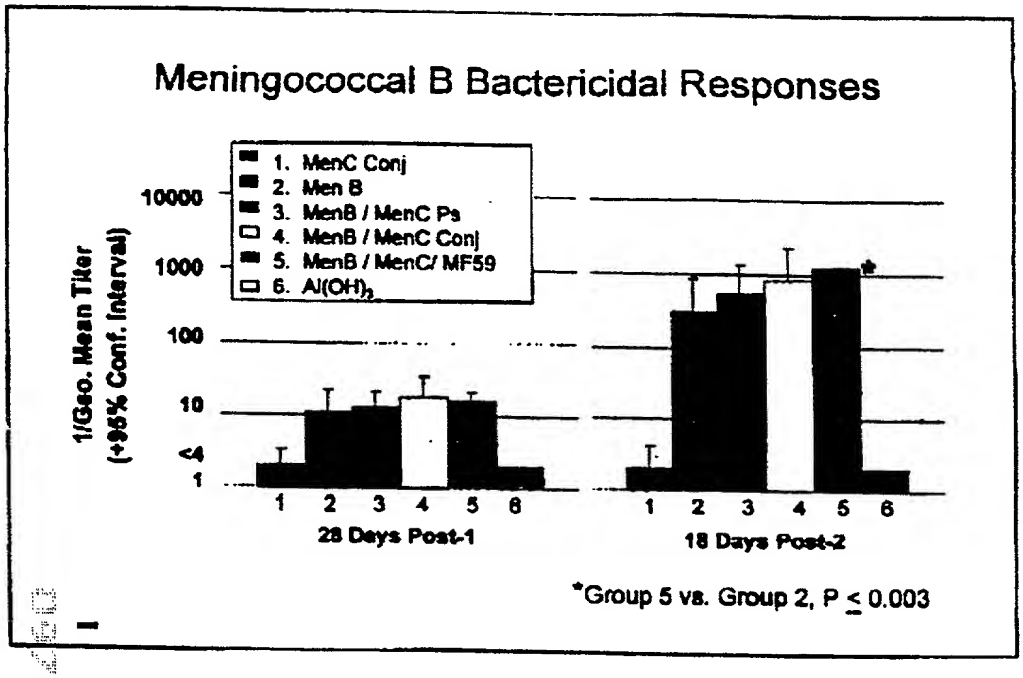

Figure 2B

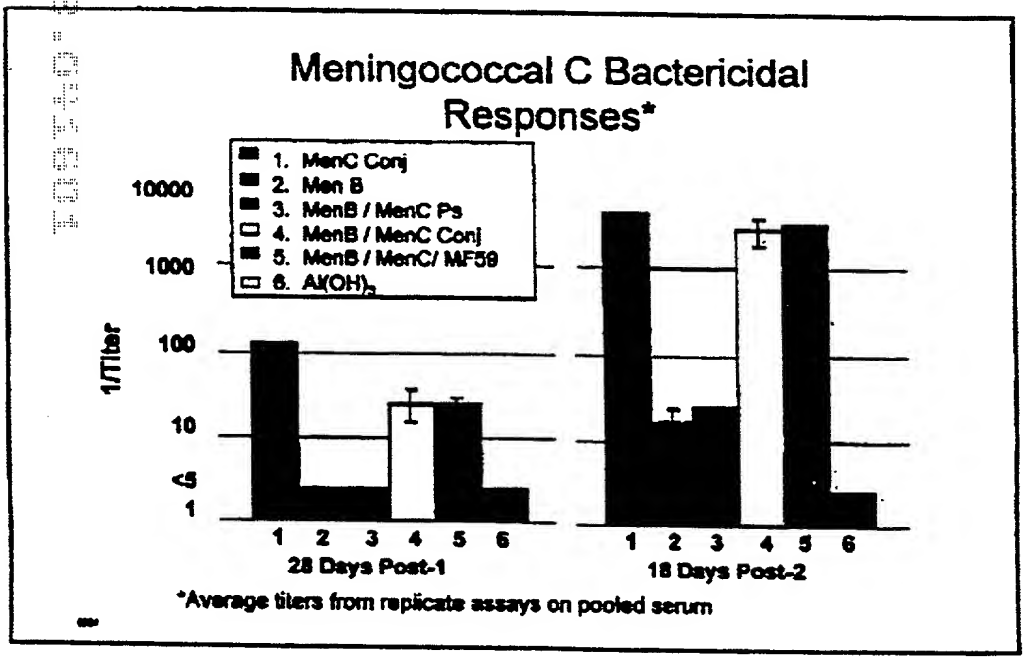

Figure 3

Ratios of Antibody Responses of Animals given Combination MenB OMV / MenC Conjugate with MF59 or $Al(OH)_3$

Assay		Ratio of GMT MF59 : GMT $Al(OH)_3$ 28 days Post - 1	18 days Post - 2
MenC	IgG	1.6	6.0**
	Bactericidal	1.0*	1.2*
MenB	IgG	0.7	1.4
	Bactericidal	0.9	1.4

*Pooled sera only tested

**$P \leq 0.001$

Figure 4

Ratios of Antibody Responses of Animals given Combination / $Al(OH)_3$ vs. Monovalent / $Al(OH)_3$

Assay		Ratio of GMT Combo : GMT Mono 28 days Post - 1	18 days Post - 2
MenC	IgG	0.5	0.5
	Bactericidal	0.2*	0.7*
MenB	IgG	1.3	1.2
	Bactericidal	1.6	2.9**

*Pooled sera only tested.

**$P \leq 0.05$

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: COMBINATION MENINGITIDIS B/C VACCINES

the specification of which (check one) __ is attached hereto _X_ was filed on May 28, 1999, as Application Serial No. IB/US99/11977, and was amended on __(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
Number	Country	Day/Month/Year Filed	Yes	No

I hereby claim the benefit under Title 35, United States Code, §120 and/or §119(e) of any United States application(s) and/or provisional applications listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status Patented, Pending, Abandoned
60/087,351	05/29/98	Abandoned
60/106,446	10/30/98	Abandoned

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States code and

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: COMBINATION MENINGITIDIS B/C VACCINES

the specification of which (check one) __ is attached hereto _X_ was filed on May 28, 1999, as Application Serial No. 09/701,453 , and was amended on __(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

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that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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